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Sex Pheromone and Blacklight Trap Studies With the Sugarcane Borer, *Diatraea Saccharalis* (F.).

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SEX PHEROMONE AND BLACKLIGHT TRAP STUDIES WITH
THE SUGARCANE BORER DIATRAEA SACCHARALIS (F.).

The Louisiana State University and Agricultural
and Mechanical College, Ph.D., 1971
Entomology

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SEX PHEROMONE AND BLACKLIGHT TRAP
STUDIES WITH THE SUGARCANE BORER
DIATRAEA SACCHARALIS (F.)

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Entomology

by
Rafael Perez Perez
B.S.A., A.M.C. University of Puerto Rico, 1957
M.S., Louisiana State University, 1964
December, 1971

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ABSTRACT

Stikem coated traps constructed from 5 gallon capacity plastic cans and baited with virgin females of Diatraea saccharalis (F.) were more effective than comparable wire traps in recapture of males. Percentages of recoveries for males released at 120', 180', and 220' from traps baited with virgin females were 18.6, 23.0, and 24.3%, respectively.

Significant differences ($p = .05$) were obtained in numbers of males attracted to traps baited only with virgin females and comparable traps containing blacklight lamps. However, differences between traps baited with virgin females plus blacklight lamps and those with blacklight lamps plus females attracted from the natural population were not significant.

Most females attracted to blacklight lamps were mated and less than 2 days old. The average number of spermatophores found in the bursa copulatrix of females attracted to blacklight was higher than that previously reported for field populations of D. saccharalis.

Arthropod predation was responsible for the low recapture of males in some experiments. Trap catches of moths were influenced by temperature and rainfall, especially their effect on reducing adhesiveness of trap surfaces.

Differentiation of pheromone and light attraction in traps was difficult and necessarily influenced by limitation on trap design. Thus, trap catches may have provided little information on sex ratios and mating behavior of natural populations of D. saccharalis.

INTRODUCTION

The struggle for life requirements between insects and man is continuous and timeless. Less than 10% of the known insect species are considered harmful; yet these pest species are man's chief competitors for health, food, and shelter. Thus, man must make use of all available methods of insect control in order to survive this struggle for existence.

During the last 3 decades, man has relied heavily on insecticides for insect control, especially the synthetic organic compounds. These have been highly efficient and have contributed significantly to better health and shelter and increases in the world's food supply, yet they have been overused and have created residue problems for man and animals. Overuse has also led to development of insecticide resistance in many pest species, and it has created public awareness to the hazards of insecticides and their impact on the environment. Thus, there is an urgent need for control systems that rely less on insecticides and more on other methods. There is a need for improvement in insect survey techniques and more research on new approaches to insect control. It may not be possible in the foreseeable future to control severely damaging insect species without insecticides, but the use of insecticides may be reduced to minimal amounts by incorporating insecticidal control with other effective methods into integrated control programs based on sound principles of pest management.

The use of light attraction for survey and control of insects dates back many years. More recently, sex attractants have been

studied for the same purpose. To date, neither approach has contributed significantly to insect control; however, both have been utilized effectively as survey techniques for some species.

The studies reported herein were undertaken with the following specific objectives in mind:

1. To determine the percent recapture of D. saccharalis males released at different distances from traps baited with virgin females.
2. To evaluate the combined effect of pheromone and light attraction for luring D. saccharalis adults from natural populations.
3. To determine the mating behavior and physiological age of females of D. saccharalis attracted to light traps.

LITERATURE REVIEW

Attraction to light traps has been used to survey insect populations and in some cases to control insects for many years. More recently, attempts have been made to use insect attractants alone or in combination with light for the same purposes. The sex attractants are often referred to as sex pheromones. A pheromone as defined by Karlson and Butenandt (1959) is a substance secreted to the outside by an animal that causes a specific reaction in a receiving animal of the same species. The term pheromone is derived from the Greek "pherein" (to carry) and "horman" (to excite).

That insect attractants may not be species specific was demonstrated with the sugarcane borer, Diatraea saccharalis (F.), when sticky traps baited with virgin females of this species attracted large numbers of males of the bluegrass sod webworm, Crambus teterrellus (Zincken), without attracting females of either species (Hammond, 1967). Males are attracted to females in most insect species, and especially in Lepidoptera.

Among insects, the attraction of one sex to the other has not always been differentiated from attraction to light, especially when lights have been used in traps that employ a sticky substance or some mechanical means that permits those caught to remain alive. Light traps in many studies have become sex attractant traps by permitting entrapped living individuals of some species to lure the opposite sex. Catches from this type of trap, especially of Lepidoptera, often contain more males than females within individual species. This combined

effect has led to problems in estimating numbers and sex ratios of natural populations from light trap catches.

Trap lanterns were patented and used quite extensively for survey and control of Lepidopterous insects attacking cotton from 1860 to 1888. Slingerland (1902) reported that for at least half a century cotton growers used lanterns in traps coated with a sticky substance to lure and entrap insects, but that these traps did not provide economic control. Trap lanterns were also used in studies of Crambus species by Felt (1894). He noted that more males than females were always attracted to the light traps.

Williams (1935) determined the time of activity of certain nocturnal insects, especially Lepidoptera, by using light traps. Later (1939) he reported a 4-year study of insect capture in light traps that included a general survey of the species trapped, their sex ratios, and the factors that affect sex ratios. He concluded that in Lepidoptera there is a strong tendency for more males than females to be caught in light traps.

Lawson, et al. (1963), and Gentry, et al. (1967), reported that populations of the tobacco hornworm, Manduca sexta (Johanson), and the tomato hornworm, M. quinquemaculata Haworth, were reduced 83% in a 113 sq. mile area of tobacco in North Carolina when blacklight traps were operated at a density of 3/sq. mile.

Hoffman, et al. (1966), reported on the effectiveness of blacklight traps baited with virgin females to reduce populations of M. sexta. They found that male catches from blacklight traps baited with 2-30 females/trap were much higher than catches from blacklight traps without females. These authors suggested intensive experiments

with female-baited blacklight traps in isolated areas of hornworm infestations to determine the feasibility of complete population control. However, Jones and Thurston (1970) reported the effectiveness of an area blacklight trapping program for hornworm control on tobacco in Kentucky and concluded that it did not provide economic control.

Shorey and Gaston (1965) demonstrated in laboratory experiments that the males of the cabbage looper, Trichoplusia ni (Hubner), would more often orient toward a light source than toward an air current carrying a weak concentration of T. ni sex attractant. Henneberry and Howland (1966) showed that 20 to 30 times more T. ni males were caught in light traps baited with pheromone than with unbaited traps. Henneberry, et al. (1967a), demonstrated that catches of T. ni males in baited light traps increased as the numbers of virgin females caged in traps were increased and that virgin females placed as far as 40 feet from traps increased male catch. Kishaba, et al. (1970a), reported results obtained with different types of traps and the synthetic pheromone of T. ni. Electric grid traps baited with pheromone attracted more males than standard survey light traps baited with pheromone. Grid traps baited with pheromone plus a 15 watt blacklight lamp caught 3.1 times more males than grid traps baited only with the pheromone.

Studies on insect attraction to traps with different light intensities and wave lengths have been published by Collins and Machado (1943), Harstock, et al. (1966), Stanley and Dominick (1970), Frost (1970), Sotherstrom (1970), Kirkpatrick, et al. (1970), and Steward and Lam (1970). Blacklight is generally more attractive to most species, and 15 to 400 watt intensities have usually been used in light trap

studies. Comparisons of the effectiveness of different trap designs in trapping insects have been published by Sparks, et al. (1967), Guerra and Ouye (1967), and Kishaba, et al. (1970a).

The earliest known attempt to use an insect sex attractant to reduce insect numbers was by Forbush and Fernald (1896). They caged virgin females of the gypsy moth, Porthetria dispar (L.), in traps to lure males from natural populations and thus reduce numbers of fertile eggs deposited. Although their trapping was considered a failure, it did serve to detect infestations efficiently. Collins and Potts (1932) found that males of P. dispar flew mostly in an upwind direction from traps baited with virgin females of this species. Later they discovered that males were attracted to benzine extracts of the last two abdominal segments of the females. This observation stimulated research on chemical synthesis of insect sex attractants. The authors stated that insect sex attractants could be used in locating infestations, as an aid in studying insect flight habits, and in detection and eradication work. Holbrook (1953) used gypsy moth attractant traps to detect infestations away from areas already infested and to check the effectiveness of chemical control. Holbrook, et al. (1960), reported on different aspects of the gypsy moth trapping program in the northeastern states and stated that air movement was probably the most important factor in trap placement.

Maksimovic (1964) discussed the relationship between numbers of egg masses deposited and the numbers of males of P. dispar caught in pheromone baited traps. He found that 10 egg masses/hectare were found for every 25 males trapped and stated that populations could be

maintained below economic levels by initiating control only after 10 egg masses/hectare were found.

Although the isolation, identification, and synthesis of the P. dispar attractant by Jacobson, et al. (1960), was later shown to be incorrect (Jacobson, et al., 1970), their work made possible the investigation of new methods of insect control. During the past decade sex attractants from many other species of Lepidoptera have been identified and synthesized. These include the silkworm moth, Bombyx mori (L.) (Butenandt, et al., 1961); cabbage looper, Trichoplusia ni (Hubner) (Berger, 1966); pink bollworm, Pectinophora gossypiella (Saunders) (Jones, et al., 1966); fall armyworm, Spodoptera frugiperda (J. E. Smith) (Sekul and Sparks, 1966); false codling moth, Argyroplote leucotreta Meyrick (Read, et al., 1968); greater wax moth, Galleria mellonella (L.) (Roller, et al., 1968); red banded leaf roller, Argyrotaenia velutinana (Walker) (Roelofs and Arns, 1968); oriental fruit moth, Grapholitha molesta (Busck) (Roelofs, et al., 1968); codling moth, Carpocapsa pomonella (L.) (McDonough, et al., 1969); and the European corn borer, Ostrinia nubilalis (Hubner) (Klum and Brindley, 1970). Research investigations are underway to determine the feasibility of using many of these for control; however, none have yet provided economic control of natural insect populations.

One of the methods commonly used to evaluate the effectiveness of light and/or sex attractant traps in a given area is to evaluate trap catches of insects that have been marked and released. Johnson (1969) pointed out some of the disadvantages of using the mark-release-recapture method for studying insect dispersal and stated that in most cases only a small percent of the released population is recovered.

Henneberry, et al. (1967b), reported that recapture of T. ni marked males averaged 17% when 22 blacklight traps baited with virgin females were placed at equal distances from each other in an experimental area of 690 acres. Kishaba, et al. (1970b), released marked males at 350, 500, and 1000 feet downwind from a synthetic pheromone source and recovered 30, 19, and 6% of those released at those distances, respectively. When 4 traps were placed in a "line" 650 feet apart, 35, 15, 5, and 2%, respectively, were recovered when males were released 160, 650, 1325, and 2020 feet downwind from the traps, and 94% of the recaptured males were recovered at the closest trap.

Wong, et al. (1971), released males of the red banded leaf roller, A. velutinana, in an apple orchard and recorded the average percent recapture in 5-8 traps, each baited with 10 virgin females and spaced 50 meters apart within the release area. Of 1015 males released, 37% were recovered. These investigators also showed that the pattern of activity of laboratory-reared males was similar to that of males from the natural population.

Techniques and dye compounds used for marking Lepidoptera for release in the field have been published by Vail, et al. (1966), Graham and Mangum (1971), and Wong and Cleveland (1970).

Gehring and Madsen (1963) developed a technique for classifying the relative reproductive age of females of the codling moth, C. pomonella, into 3 groups as follows: those females with abdomens replete with ovaries and fat bodies or that were nearly replete with only a small cavity in the anterior portion were classified as group 1 or 1 day old and assumed to have not deposited eggs; those with abdomens from 1/4 to 3/4 filled with ovaries and fat bodies were

classified as group 2 and assumed to be 2-4 days old; those with atrophied ovaries and no or little fat bodies were classified as group 3 and assumed to be more than 4 days old. Using this technique, the authors determined that most females of C. pomonella caught in blacklight traps were from 1 to 4 days old and that very few were unmated.

Tomlinson (1966) used Gehring and Madsen's technique in a two year study of females of the cranberry fruitworm moth, Acrobasis vaccinii Riley, caught in blacklight traps and determined that 90% were 1-4 days old and that 3.5% were unmated. Hendrix, et al. (1970), collected females of the tobacco budworm, Heliothis virescens (C. F. Smith), and cotton bollworms, H. zea (Boddie), from 7 blacklight traps located in cotton for a period of 6 months and determined the numbers of matings/female according to a spermatophore count technique developed by Callahan (1958). Most mated individuals in both species had mated only once; however, some had mated as many as 6 times. Thirty-five percent of the cotton bollworm females and 27% of the tobacco budworm females were mated. Gentry, et al. (1971), reported that the percent mated tobacco budworms captured in blacklight traps in north Florida were 57, 73, and 59% for the years 1967, 1968, and 1969, respectively. Multiple matings among mated moths averaged 64, 55, and 18% for these years, respectively. For cotton bollworms, the incidence of mated females was 37, 37, and 46% for the years 1967, 1968, and 1969, respectively, and multiple mating occurred less frequently than in H. virescens.

Perez and Long (1964) discovered a potent sex attractant in the sugarcane borer, Diatraea saccharalis (F.), in 1963. Females began to emit the attractant soon after emergence and were most attractive

during the first 3 days of life, after which attractiveness decreased with age. Females gradually ceased to attract males after mating, and most females mated only once in nature. Extracts from virgin female abdomens prepared in benzene or methylene chloride were effective for only a few hours in attracting males when exposed to the environment on filter papers. They also found that mating activity of this species occurred mostly between the hours of 1:00 to 4:00 A.M.

Hammond (1967) showed that virgin female moths of D. saccharalis could be used to achieve significant reductions in borer damage in small field plots when sticky traps, each baited with one virgin female, were maintained continuously in plots at the rate of 400 to 800 traps/acre. He recovered only 3.89% of 950 males which had been released 25-50 feet from 2 sticky traps, each baited with 2 virgin females.

Patrick and Hensley (1970) recovered only 4.44% of 1936 marked males of D. saccharalis released downwind at distances ranging from 40 to 320 feet from a sticky trap baited with 10 virgin females. Differences in numbers of males recaptured at points of release situated at different distances from the trap were not statistically significant ($p=.05$). Interference from attractive females of the natural population might not have been an important factor, since only 132 unmarked males were captured during the two week duration of the experiment. The authors believed that dispersion of released males away from the release trap-area before they became responsive to the sex attractant was mostly responsible for the low percentage of recapture.

Hammond and Hensley (1970) developed a bioassay technique for detecting active fractions of the D. saccharalis attractant, and

Tribble (1966) conducted purification and structural analysis experiments. However, the attractant has not yet been identified and synthesized.

MATERIALS AND METHODS

Recapture of Laboratory Reared Male Sugarcane Borers

Field Trapping Area. Tests to determine the percent recapture of marked males released at different distances and directions from traps baited with virgin female sugarcane borers were conducted during 1970 and 1971 at the Louisiana State Experiment Station farm near St. Gabriel, Louisiana. This site was selected because it afforded less interference from natural sugarcane borer infestations than many other local areas of sugar production. Sugarcane was not planted there until 1969; thus, there was little if any endemic sugarcane borer infestation present. Uninfested seed cane was planted in 1969, and insecticides were applied on rigorous fixed application schedules to minimize development of infestations. Furthermore, there is no other sugarcane grown within a 5 mile radius of the St. Gabriel farm, thus reducing the chance of moth migration from other sugarcane farms.

The recapture experiments were conducted within a 20 acre planting of single seedling entries in a varietal selection trial. Since these seedlings were randomly distributed in the test, there was potentially little if any varietal effect on the moth recapture experiments. The seedlings were planted 36" apart, and each produced from 3 to 30 mature stalks when harvested. There was no other cane grown on the St. Gabriel farm in 1970, and in 1971 there was a similar sized block grown approximately 1/2 mile from the experimental area.

Laboratory Techniques for Rearing and Holding Moths Prior to Use in the Field. The sugarcane borer moths were obtained by rearing

larvae on a Pinto bean diet according to procedures described by Hensley and Hammond (1968). The pupae were sexed with the aid of a binocular microscope and males and females kept in separate but similar cages until emergence. Each emergence cage consisted of a 1-gallon capacity cylindrical ice cream carton from which the top had been removed and replaced with a 64-mesh plastic screen. The pupae were held within a cage in a 3" diameter petri dish which rested on a 2" layer of moist vermiculite. Moths that emerged were removed daily and placed in holding cages similar to those used during emergence, except that they did not contain vermiculite. Only 1-day old males were used in the release-recapture tests, and 1-2 day old virgin females were used as bait moths in field traps. All rearing work preparatory to releases of moths in the field was conducted in a laboratory room at a temperature of $76^{\circ} \pm 4^{\circ}$ F.

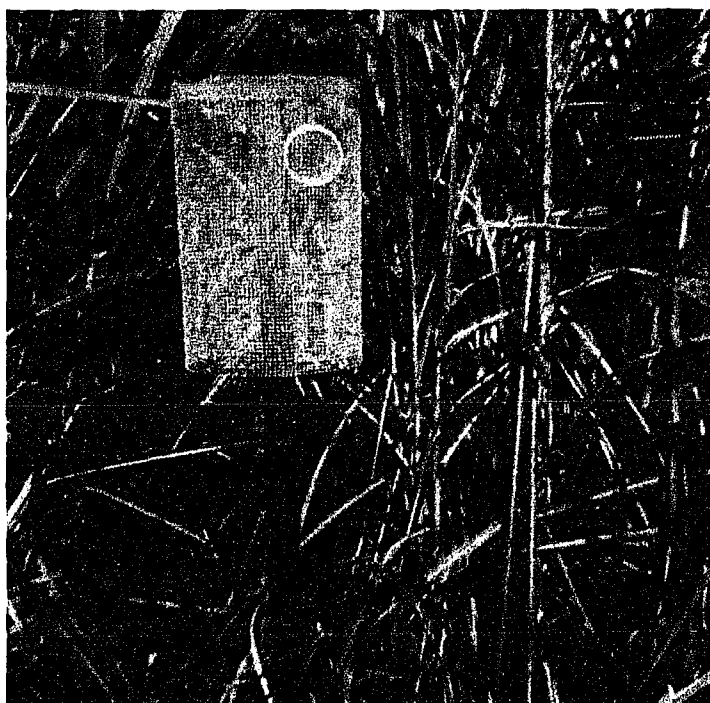
Male moths to be released were marked in the laboratory with Black Ray^R Fluorescent dyes (Ultraviolet Products Company, Inc., St. Gabriel, California). Different colored dyes were employed to identify males released at different times or distances from traps. Immediately before carrying males to the field for release, 1/4 teaspoon of powdered dye was placed in a holding cage containing the males. The cage top was then covered with a paper towel and air puffed inside with a bulb-type pump to aid in coating the moths with dye. The dye adhered to the integument of the moths and was especially noticeable on the wings and antennae. After recapture, the marked moths were differentiated from those trapped from natural populations by detecting the presence of dye on the integument with the aid of a binocular microscope and fluorescent lamp. The dye was easily detected on recaptured

males for a period of 3 days after release. Cages of males marked in this manner were transported to the field in 25 lb. capacity paper bags, where they were released by opening the cages and shaking them gently over sugarcane plants. All releases were made between the hours of 5:00 and 7:00 P.M. Caged females used as bait moths were transported to the field in paper bags similar to those used for transporting males.

Pheromone Traps. The traps initially employed in the male recapture experiments were similar to those used by Patrick (1968). Each trap (Figure 1-A) consisted of a cylinder of 1/4" hardware wire screen, 18" long and 12" in diameter. The exterior surface of the trap was coated with Stikem^R (Michael Pelton Company, Oakland, California), an adhesive substance which served to entrap males lured to the trap. The cylindrical trap was suspended in a vertical position in the field by attaching it to a 2" x 2" x 6' redwood stake which was driven into the soil. A 2" x 2" x 12" piece of wood nailed across the top of the trap was used to attach traps to stakes.

The hardware wire screen traps were replaced with those made from plastic cans after comparison of the two types indicated that more males were recaptured in the plastic can traps. These traps (Figure 1-B) were constructed from 5 gallon capacity white plastic cans from which the tops and bottoms had been removed. The inner surface of each trap was coated with Stikem and recoated at 3-day intervals during each test. Two types were used during the remainder of the recapture studies. One type (Figure 1-B) was composed of the entire cylindrical surface area of a can, and the other (Figure 2-A-B) was composed of 1/2 of the cylindrical area. Both types of plastic can traps were

Figure 1. Wire trap (A) and plastic can trap (B) used in the recapture of males of the sugarcane borer.

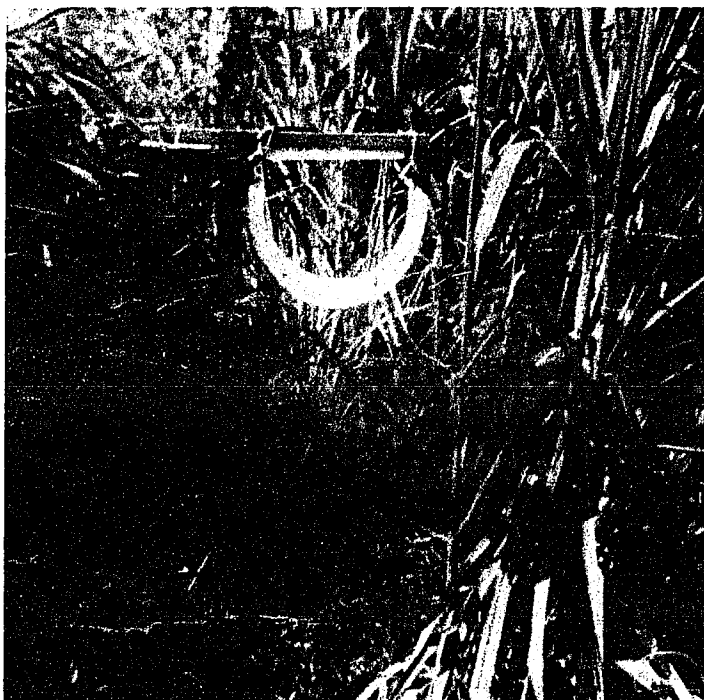


A

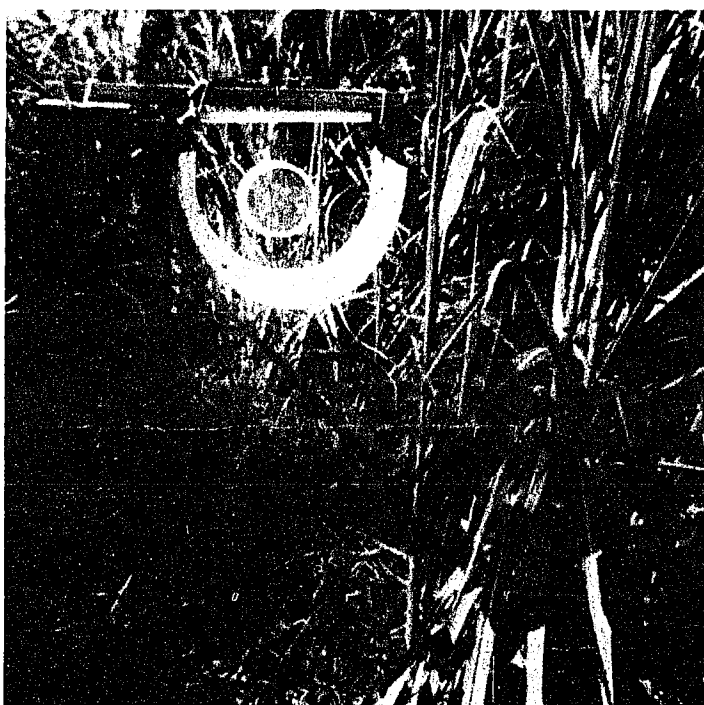


B

Figure 2. Types of traps: (A) unbaited blacklight trap. (B) Black-light trap baited with caged females.



A



B

suspended in a horizontal position in the field by attaching them to stakes similar to those used to support the hardware screen traps. All traps were suspended approximately 4 1/2' above ground level regardless of the height of sugarcane stalks. Trap stakes were coated with a 3" band of Stikem to prevent ants from destroying the bait moths caged within traps.

Five virgin female sugarcane borers were used in each trap as bait, regardless of trap design. They were confined in a cylindrical 1/2 pint capacity ice cream carton from which the top and bottom had been removed and replaced with 12-mesh copper wire screen. The moth cage was suspended in the trap by a wire extending from it to the top of the trap.

Male Recapture Experiments. Experiment 1 was conducted during the last week in June, 1970, when the sugarcane crop averaged approximately 18" in height. Twelve baited hardware screen traps were arranged 18' apart in a circle with a radius of 120 feet, and marked males were released daily for 4 days from a central point in the circle. The numbers of marked males recaptured and the numbers of males trapped from the natural population in individual traps were recorded daily after males were first released.

Experiment 2 was conducted from July 7 to July 16. Two sets of hardware wire traps, each consisting of 6 traps, were located 120' apart in the field. Individual traps in each set were spaced 18' apart and arranged in a line extending from northwest to southeast. Males were released on 5 different dates from July 7 to July 16 at a central point between the two lines of traps. The number of males recaptured

and those trapped from the natural population was recorded for individual traps the morning after each release.

Experiment 3, which was conducted from July 27 to August 4, was designed to compare the efficiency of the hardware wire traps with that of traps constructed from plastic cans. Three wire traps and 3 can traps were spaced 18' apart and arranged in a paired block design in a line extending from northwest to southeast. Marked males were released in a northeastern direction at a point located 60' from the center of the line of traps. Males were released on 5 different dates, and records were kept of the number recaptured in each trap.

Experiment 4 was conducted to obtain information on the percent recapture when males were released simultaneously at two different points northeast of a line of traps. Six can traps were arranged 18' apart in a line extending from northwest to southeast. All males available on each release date were separated into 2 groups, and each group was coated with a different colored dye. The two groups of moths were released 120' and 180', respectively, from the line of traps on each release date. Five releases were made from August 6 to August 16, and the number of males recaptured was recorded the morning after each release. During the time when releases were made, the sugarcane crop averaged about 5' in height.

Experiment 5 was conducted to determine the percent recapture when marked males were released 220' northeast of a line of 6 traps baited with virgin females. This test was conducted in June, 1971, at the St. Gabriel farm. The first year stubble crop of the 20 acre varietal planting previously described was used as a study area. During June the height of the sugarcane in this varietal planting averaged about

24". The pheromone trap employed was similar in all respects to the plastic can trap used in previous tests, except that only one-half of a 5-gallon can was used as a trapping surface to entrap moths. Six of these traps were positioned in the field 24' apart and aligned from northwest to southeast. From June 1 to June 30, 9 releases of marked males were made at a point located 220' northeast of the traps. Records were kept of the number of males recaptured and those trapped from the natural population.

Comparison of Pheromone and Blacklight Traps for Attracting Adult Sugarcane Borers from a Natural Population

Field Trapping Area. Experiment 6 was conducted in September and October, 1970, at Westover Plantation near Rougon, Louisiana. Approximately 1600 acres of sugarcane are in production annually at Westover, and insecticides are applied for control of economically damaging sugarcane borer infestations in July and August. However, no insecticides are applied after August 31, and fall infestations are usually heavy, especially in the tops of stalks, where the larvae cause little if any economic damage.

A 25 acre field of sugarcane variety C.P. 43-47, first year stubble crop, located near the center of the plantation was selected as a trapping area. This field had received 2 applications of azinphos-methyl, applied at a rate of .75 lb. ai/acre/application for sugarcane borer control in July and August; however, when this test was initiated on September 19, more than 25% of the stalks contained larvae in the leafsheaths of the topmost internodes. Traps were located in a 1200 sq. ft. area in the center of this field.

Experimental Design. A complete randomized block experimental design with treatments replicated 3 times was employed. Treatments consisted of the following 3 types of traps: (1) Pheromone, (2) Blacklight + entrapped females from the natural population, and (3) Pheromone and blacklight in combination. The distance between traps within the trapping area was 300'.

Types of Traps. The basic components of all 3 types of traps, i.e., stakes, plastic trapping surface, and mounts were identical and similar to that shown in Figure 2-A-B. For the blacklight trap, an F6B5 blacklight bulb was positioned laterally 9" above the center of the plastic trapping surface. This bulb was connected with an electrical wire to a transformer which was wired to a 12 volt battery located on the ground. An automatic switch which shut off the electrical current during daylight hours was located within the transformer; thus, the light was in operation only between sundown and daybreak. The pheromone trap was similar to that shown in Figure 2 and contained 5 virgin females as bait. The pheromone-blacklight trap (Figure 2-B) contained a blacklight bulb and 5 caged virgin females positioned 6" above the trap surface, whereas the blacklight trap (Figure 2-A) contained a similar bulb plus those females attracted to it that became entrapped in the Stikem.

Trapping Procedure. The traps were serviced after 5:00 P.M. Bait cages containing virgin females less than 2 days old were placed in the pheromone, and pheromone-light combination traps and the darklight bulbs, transformers, and batteries were checked to assure that they were functioning. All trap surfaces were coated with Stikem. Each morning after the traps were in operation, the sugarcane borer moths

caught on each trap were placed in a vial containing xylene and returned to the laboratory where they were transferred to 95% alcohol and held for subsequent study. Traps were operated from September 19 to October 4, 1970, and during that time the height of the sugarcane stalks in the trapping area averaged about 7'.

Reproductive Status and Physiological Age of Females Attracted to Blacklight Traps (sex ratio of catch). All moths trapped were examined with the aid of a binocular microscope to determine the numbers of males and females attracted to different types of traps. Mating incidence of females was determined by counting the number of spermatophores found in the bursa copulatrix according to the techniques developed by Callahan (1958). Physiological age of the females was determined by examining the abdominal condition of the female according to methods described by Gehring and Madsen (1963).

RESULTS

Recapture of Laboratory Reared Male Sugarcane Borers

Experiment 1. The numbers of male sugarcane borers recaptured when males were released from a central point within a 120' diameter circle of traps baited with virgin females is shown in Table 1. The number recaptured was extremely low regardless of direction of individual traps from the release point. Only 5.3% of 297 males released on 4 dates were recaptured. Percentages recaptured on different dates of release ranged from 7.8% on July 26 to 0% on July 27. The percentage recapture of marked males was too low in this test to provide meaningful information on any possible effect of wind direction on trap catch. Only 5 males were trapped from the natural population of sugarcane borers.

Experiment 2. Table 2 shows the number and direction of recapture when males were released between two lines of traps baited with virgin females and positioned crosswise (northwest to southeast) to the direction of prevailing winds (southwest to northeast) at the time of release. Upwind traps, those positioned southwest of the release point, captured more males than downwind traps (those positioned northeast of the release point). However, differences in numbers recaptured were not statistically significant between upwind and downwind traps. Of 1015 marked males released on 5 different dates, 113 (11.7%) were recaptured in upwind traps and 30 (2.9%) were recaptured in downwind traps. Only 6 males were trapped from the natural population during the time when males were released (July 7-16).

Table 1. Number, percent, and direction from release site of recaptured male sugarcane borers released from a central point within a 120' diameter circle composed of 12 sticky traps, each baited with 5 virgin females. St. Gabriel, Louisiana, June 25-29, 1970.

Date of Release	Number of Males Released	Number Recaptured					Percent Recaptured	Number of Males Trapped From Natural Population
		Direction of traps from release point						
		North	South	East	West	Total		
June 26	133	1	4	2	5	12	7.8	2
June 27	33	0	0	0	0	0	0	0
June 28	67	1	0	1	1	3	4.4	0
June 29	64	0	1	0	0	1	1.5	3
Total	297	2	5	3	6	16	5.3	5

Table 2. Effect of prevailing wind direction on recapture when male sugarcane borer moths were released at a centrally located point between 2 lines of baited pheromone traps extending from northwest to southeast. St. Gabriel, Louisiana, July 7-16, 1970.

Date of Release	Number of Males Released	<u>Number and Percent Recapture</u>				Number of Males Trapped From Natural Population
		Upwind Traps		Downwind Traps		
		No.	%	No.	%	
July 7	345	62	18.0	12	3.5	0
July 9	120	19	15.8	4	3.3	2
July 10	180	6	3.3	2	1.1	0
July 14	140	2	1.4	6	4.4	0
July 16	230	24	10.4	6	2.2	4
Total or %	1015	113	11.7	30	2.9	6

Experiment 3. A comparison of the number of males recaptured in 3 "hardware wire traps" with those recaptured in 3 "plastic can traps" is shown in Table 3. Greater numbers of males were recaptured in the "can" traps than in "wire" traps. From a total of 587 males released on 5 dates, 14 (2.4%) were recaptured in "wire" traps compared to 54 (9.6%) in "can" traps. Differences in trap catches between "wire" and "can" traps were statistically significant ($p=.01$). Fifteen males from the natural population were recaptured on "can" traps compared to 2 on "wire" traps.

Experiment 4. The number of males recaptured when releases were made 120' and 180' downwind from a line of can traps is shown in Table 4. Sixty-five of 343 (18.6%) and 67 of 291 (23.0%) were recaptured when releases were made at 120' and 180', respectively. These differences were not statistically significant ($p=.05$). Percentages of recapture on individual release dates, which ranged from 8.3% to 45.0% for males released at 120' and 11.1% to 31% for those released at 180', were higher than that obtained in previous experiments. A total of 40 males were trapped from the natural populations.

Experiment 5. Table 5 shows the number of males recaptured when releases were made 220' downwind from a line of 6 "half-can" traps. Of 683 males released from June 1 to June 30, 1971, 166 (24.3%) were recaptured. Percentages of recapture on individual dates of release were comparable to those obtained in Experiment 4 and ranged from 11.4% to 31.4%. A total of 144 males were trapped from the natural population.

Table 3. Comparison of the number of male sugarcane borers recaptured in 3 "wire" traps and 3 "can" traps. St. Gabriel, Louisiana, July 27-August 4, 1970.

Date of release	Number released	Wire traps			Can traps		
		Total no. recaptured	%	Total no. trapped from natural pop.	Total no. recaptured	%	Total no. trapped from natural pop.
July 27	110	0	0.0	0	9	8.2	3
July 29	79	2	2.5	0	4	5.1	0
August 2	170	12	7.1	2	23	13.6	3
August 3	92	0	0.0	0	7	7.6	3
August 4	136	0	0.0	0	11	8.1	6
Total or %	587	14	2.4	2	54	9.4	15

Table 4. Number of male sugarcane borers recaptured when releases were made 120' or 180' downwind from a line of 6 traps, each baited with 5 virgin females. St. Gabriel, Louisiana, August 6-18, 1970.

Date of Release	Distance From Release Point						Number Trapped From Natural Population
	120'			180'			
	Number Released	Number Recaptured	Percent	Number Released	Number Recaptured	Percent	
August 6	120	10	8.3	90	17	18.8	5
August 8	60	9	15.0	35	4	11.4	8
August 9	40	18	45.0	43	8	18.6	4
August 10	33	12	36.6	33	10	30.3	12
August 16	90	16	17.7	90	28	31.1	11
Total or %	343	65	18.6	291	67	23.0	40

Table 5. Number of male sugarcane borers recaptured when releases were made 220' downwind from a line of 6 "half-can" traps, each baited with 5 virgin females. St. Gabriel, La., June 1-30, 1971.

Date of release	Number released	Number recaptured	%	No. males trapped from natural pop.
June 1	70	8	11.4	7
June 2	51	16	31.4	2
June 4	31	5	16.1	4
June 6	141	44	31.2	10
June 7	106	31	29.3	30
June 9	70	16	22.9	34
June 21	115	24	20.9	20
June 24	49	9	18.8	24
June 30	50	13	26.0	4
Total or %	683	166	24.3	144

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Experiment 6. The numbers of male sugarcane borers caught in traps baited with (1) 5 virgin females, (2) blacklight lamps plus females from the natural population, and (3) a combination of these two treatments is shown in Table 6. Greater numbers of males were caught in treatments 2 and 3, i.e., those that included blacklight lamps. Differences between numbers of males caught in these 2 treatments were not statistically significant ($p=.05$), but male catch in each treatment was significantly higher ($p=.05$) than that obtained in treatment 1, i.e., traps baited only with virgin females.

The numbers of females caught in treatments 1, 2, and 3 are shown in Table 7. No female was caught in traps baited only with virgin females (treatment 1), and 24 and 35, respectively, were caught in treatments 2 and 3, i.e., those that included blacklight lamps (Table 7).

Reproductive Status and Physiological Age of Females Attracted to Blacklight Traps (sex ratio of catch)

These data are shown in Table 8. Only two of the females caught were unmated. The bursa copulatrix of each of 32 females contained 1 spermatophore, and 21 and 4 contained 2 and 3, respectively.

More than one-half (33) of the females caught were classified as belonging to the youngest age group (1); and 24 and 2, respectively, were found to belong to age groups (2) and (3).

The sex ratio of trapped moths was found to be 1 female for every 9.8 males trapped.

Table 6. Numbers of male sugarcane borers caught in traps baited with (1) virgin females, (2) blacklight lamps + females attracted from the natural population, and (3) virgin females + blacklight lamps + females attracted from the natural population. Westover Plantation, Rougon, Louisiana. September 19-October 4, 1971.^a

		<u>Numbers trapped from natural population</u>			
Treatment no.	<u>Replication number</u>				
(Type of trap)	1	2	3	Total	Mean ¹
1	45	44	51	140	46.6 ^a
2	97	90	89	276	92.0 ^b
3	93	120	93	306	102.0 ^b

¹ Means not followed by the same letter differ significantly at the 5% level of probability according to Duncan's multiple range test (LeClerc, 1957).

^a Based on data shown in appendix Table 4. Analysis of variance shown in appendix Table 5.

Table 7. Numbers of females attracted to traps baited with (1) virgin females, (2) blacklight traps + females attracted from the natural population, and (3) virgin females + blacklight traps + females attracted from the natural population. Westover Plantation, Rougon, Louisiana, September 19-October 4, 1970.

Date	Number/trap								
	Treatment no. (Type of trap)								
	(1)			(2)			(3)		
	Rep. number			Rep. number			Rep. number		
	1	2	3	1	2	3	1	2	3
9/19	0	0	0	1	1	0	0	1	0
9/20	0	0	0	3	0	1	2	4	4
9/27	0	0	0	3	4	5	5	5	6
10/2	0	0	0	1	1	0	0	0	0
10/3	0	0	0	0	0	0	1	1	0
10/4	0	0	0	0	2	2	2	3	1
Total/trap	0	0	0	8	8	8	10	14	11
Total	0			24			35		

Table 8. Reproductive status and physiological age of female sugarcane borers and sex ratio of catch from 6 traps with blacklight lamps. Westover Plantation, Rougon, Louisiana, September 19- October 4, 1971.¹

Total Number Caught	Number with 0, 1, 2 or 3 Spermatophores ² in the Bursa Copulatrix ²				Number in Physiological Age ³ Groups 1, 2, and 3 ³			Sex Ratio of Trapped Moths Females to Males
	(0)	(1)	(2)	(3)	(1)	(2)	(3)	
59	2	32	21	4	33	24	2	1:9.8

¹ Figures are total numbers trapped in 6 traps.

² Determined according to techniques developed by Callahan, 1958.

³ Age groups are categorized from youngest (1) to oldest (3) according to the age classification described by Gehring and Madsen (1963).

DISCUSSION

The percent recapture of the males in the first experiment conducted (Table 1) was very low, and useful information was not obtained concerning the influence of prevailing wind on the orientation of males to virgin females. The low number of males recovered in this test was most likely due to (1) cool temperature, (2) arthropod predation, and (3) inefficient capture in the wire traps employed. This experiment was conducted in June when night temperatures were often below 15° C. The males were released in large groups on plants near ground level, and they were sluggish and not prone to fly. Ants, spiders, and carabids were observed preying on the released males, and numerous dyed remnants of wings were found the day after the releases were made. Inefficient capture in the wire traps may have been a factor, especially since percent recapture was much higher in can traps used in subsequent experiments. Males were always released in lower numbers/plant and at sites higher on plants in later experiments in order to minimize arthropod predation.

Differences between the number of males recaptured in upwind and downwind traps (Table 2) were not significant ($p=.05$), although upwind traps captured almost 4 times (79.1%) as many males as downwind traps. Furthermore, male recovery in upwind traps was higher on 4 of the 5 occasions when releases were made. Since wind direction was recorded only at the time when males were released, it could have changed on the one occasion (July 14) when more males were recovered in the downwind traps. The high percent recovery of the moths released upwind from the

traps also lends credence to the hypothesis that pheromone attraction is associated with wind direction (Tables 3, 4, and 5).

Five gallon can traps were found to be more efficient in capturing released males than wire traps of about the same dimensions (Table 3). Rapid drip-off of the Stikem from trap surfaces apparently greatly affected the ability of the wire trap to retain moths alighting on the traps. Furthermore, the unprotected trap surface of the wire traps appeared to be more affected by rain and dew than the protected surfaces of the can traps. Films of moisture which lowered the adhesive capacity of the Stikem coating were found more frequently on wire traps than on can traps. Although the Stikem on the inner surfaces of the can traps also tended to drip from the lateral walls, it accumulated in the bottoms of the traps and little drip-off was observed. The concentration of Stikem on the lower surfaces of traps probably accounts for the majority of moths being caught on lower trap surfaces. Although the entire trap surface of can traps was recoated at 3 to 4 day intervals, few moths were caught on the upper trap surfaces at any time. Sharma, et al. (1971), used cylindrical solid surface traps in recapture studies with T. ni and reported that 91% of the moths recaptured were caught in the lower surfaces of the traps. Nevertheless, it is possible that the pheromone substance in vapor form is heavier than air and thus was responsible for most of the males being caught on lower trap surfaces. Killinen and Ost (1971) observed on still nights that males of T. ni approached on a low flight trajectory and gradually rose to a pheromone source.

The 9.4% recapture of D. saccharalis males in can traps (Table 3) was much higher than the 3.98% recapture reported by Hammond (1967) in

smaller can traps and the 4.44% recapture reported by Patrick (1968) in wire traps. Hammond (1967) released males between 6:00 and 11:00 A.M., and Patrick (1968) released them between 3:00 and 4:00 P.M. All releases made in this study were between 5:00 and 7:00 P.M. Although much improvement was made in recapture percentages, it is still possible that percent recapture could be further improved by releasing males during the time (midnight to dawn) when they are most responsive to the pheromone. Patrick and Hensley (1970) reported that dispersion away from release points prior to males becoming responsive to the pheromone was a factor in reduced trap catches.

Differences in percent recapture between release points in Table 4 (18.6 and 23.0 for males released at 120' and 180' from virgin female traps, respectively) were not statistically significant ($p=.05$). However, they were much higher than the percent recapture (4.44) obtained by Patrick and Hensley (1970), who released males at distances ranging from 40' to 320' from virgin female traps. Dispersion of moths from release points prior to their becoming responsive to the pheromone may have been responsible for the lack of significance between numbers recaptured from different release points.

During the studies at St. Gabriel farm in 1970, only 68 males from the natural population were captured from June to mid-August, thus providing evidence that natural populations did not interfere to any extent with recapture of released males. Further evidence of the low incidence of infestation in the St. Gabriel farm cane crop was provided by infestation surveys. Larval infestation during that period of time never exceeded 5%, thus no insecticide was required to prevent economic crop damage.

Comparisons of the numbers of males attracted to different types of traps (Table 6) show that significantly fewer males ($p=.05$) were attracted to virgin female traps than those containing lights plus virgin females or lights plus only those females trapped from the natural population. However, no significant difference was found between numbers of males caught in the last two types of traps mentioned. This phenomenon only serves to point out the difficulty of attempting to separate pheromone effect from light effect, i.e., a "light" trap also becomes a pheromone trap once female moths are attracted and retained alive in the trap.

Most females removed from light traps were mated and less than 2 days old (Table 8). Their physiological age further substantiates the fact that they greatly enhanced attraction in "light" traps. Perez and Long (1964) found D. saccharalis females to be most attractive to males in the first 2 days of life.

The ratio of males to females caught in blacklight traps in this study was 1 to 10. Steward, et al. (1967), also reports a low ratio of about 1 female to 6 males in blacklight trapping experiments with tobacco hornworm, M. sexta.

Perez and Long (1964) found a ratio of 5.4 females to 4.6 males in catches of D. saccharalis in a large walk-in light trap located in the Hill Farm at Louisiana State University. Hammond (1967) found a ratio of 1 female to 10 males in D. saccharalis catches in a dissimilar type of light trap. These observations only serve to emphasize that sex ratios of Lepidoptera caught in light traps vary greatly depending on design of the trap and that light traps probably have little value in determining the sex ratio of natural populations of insects.

Of 59 D. saccharalis females caught in light traps in this study, 32 had mated once and 21 twice and 4 three times based on the number of spermatophores found in the bursa copulatrix of each female trapped (Table 8). Thus, light traps may not reflect the true reproductive status of field populations, as observations of moths shaken from sugarcane plants in the field indicate a much lower incidence of multiple mating than that found in this study (Hensley, personal communication).

CONCLUSIONS

1. Cylindrical can traps coated with Stikem and baited with virgin females recaptured more D. saccharalis males than comparable traps constructed from hardware wire.

2. About 24% of 1 day old males released 220' from a trap baited with virgin females of D. saccharalis were recaptured.

3. Catches of D. saccharalis males are increased when blacklights are placed with attractant females in traps.

4. Traps containing blacklight lamps and baited with 5 virgin females caught 1.1 times more D. saccharalis moths than comparable unbaited traps.

5. A ratio of 1 female to 10 males was found in D. saccharalis catches from traps containing blacklight lamps plus 5 virgin females plus those females attracted to the light from the natural population.

6. Most females attracted to blacklight lamps are mated and less than 2 days old.

7. The average number of spermatophores found in the bursa copulatrix was higher for females collected from blacklight traps than that previously reported for field collected females.

8. Arthropod predation can be responsible for low recapture of released males.

9. Differentiation of light from pheromone attraction in traps is difficult and necessarily influenced by limitations on trap design, thus trap catches may provide little information on sex ratios or mating behavior of natural populations of D. saccharalis.

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VITA

Rafael Perez Perez was born January 28, 1928, at Yauco Puerto Rico and received his elementary and high school education from Holy Rosary School, where he graduated in 1945. From 1945 to 1947 he was enrolled at the University of Puerto Rico and left to become a provisional school teacher in the rural area of Southern Puerto Rico.

In 1951 he entered the U.S. Army and was honorably discharged in 1953. He then reentered the University of Puerto Rico and received a B.S. degree in Agriculture in 1957. After working for the private industry, he was employed in 1958 by the Agricultural Experiment Station of the University of Puerto Rico to do horticulture research on coffee. In 1962 he was granted leave to pursue graduate degree training at Louisiana State University and received the M.S. degree in Entomology in 1964. He worked as a research entomologist at Fortuna Substation, of the Agricultural Experiment Station of the University of Puerto Rico, from 1964 to 1969 and then reentered Louisiana State University, where he is currently a candidate for the Ph.D. degree.

Rafael was married to Paquita Roura in June, 1958, and they have two sons, Rafael and Arnaldo. He is a member of Gamma Sigma Delta, Sigma Xi, and the Louisiana Entomological Society.

APPENDIX

Table 1. Student t-test analysis of numbers of marked males recaptured in upwind traps vs. downwind traps. L.S.U. Farm, St. Gabriel, Louisiana. July 7-16, 1970.

Date	Percent Recovery		Difference (a-b)
	Upwind traps (a)	Downwind traps (b)	
July 7	18.0	3.5	14.5
July 9	15.8	3.3	12.5
July 10	3.3	1.1	2.2
July 14	1.4	4.4	-3.0
July 16	10.4	2.6	7.8

$t_{.05} = 2.10$

tabulated $t_{.05} = 2.78$

Table 2. Student t-test analysis of numbers of males recaptured on 3 wire traps vs. numbers racaptured on 3 can traps. L.S.U. Farm, St. Gabriel, Louisiana. July 27-August 4, 1970.

Date	Percent Recovery		Difference (b-a)
	Wire trap (a)	Can trap (b)	
July 27	0.00	8.18	8.18
July 29	2.53	5.06	2.53
August 2	7.05	13.53	6.48
August 3	0.00	7.61	7.61
August 4	0.00	8.08	8.08

$t_{.01} = 6.25$

tabulated $t_{.01} = 4.60$

Table 3. Student t-test analysis of numbers of males recaptured 120' vs. 180' distance from 6 pheromone traps. L.S.U. Farm, St. Gabriel, Louisiana. August 6-18, 1970.

Date	Percent Recovery		Difference (a-b)
	120' (a)	180' (b)	
August 6	8.3	19.9	-11.6
August 8	15.0	11.1	3.9
August 9	45.0	18.6	24.6
August 10	36.0	30.0	6.0
August 16	17.7	31.1	-13.4

$$t_{.05} = .314$$

$$\text{tabulated } t_{.05} = 2.78$$

Table 4. Number of male sugarcane borers caught in traps baited with (1) virgin females, (2) blacklight lamp + females attracted from the natural population, and (3) virgin females + blacklight lamp + females attracted from natural population. Westover Plantation, Rougeon, Louisiana. September 19-October 4, 1970.

Date	Type of trap								
	(1)			(2)			(3)		
	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)
Sept. 19	3	4	3	7	10	3	2	12	3
Sept. 20	5	6	5	26	4	12	15	25	27
Sept. 27	17	7	29	38	47	57	44	36	44
Oct. 2	10	8	0	10	6	2	6	10	2
Oct. 3	1	6	5	9	3	1	10	14	2
Oct. 4	9	13	9	7	20	14	16	23	15
Total/trap	45	44	51	97	90	89	93	120	93
Total/treatment	140			276			306		

Table 5. Analysis of variance of number of male moths attracted to traps baited with (1) virgin females, (2) blacklight lamps + females attracted from natural population, and (3) virgin females + blacklight lamps + females attracted from natural population. Westover Plantation, Rougon, La. September 19-October 4, 1970.

Source of variation	df	SS	MS	F
Total	8	5769.56	721. 9	
Replication	2	89.56	44.78	
Treatment	2	5216.89	2608.44	22.53
Error	4	463.11	115.77	

Tabulated $F_{.05} = 19.00$

Table 6. Sex ratio of the sugarcane borer moth caught in traps baited with (1) blacklight lamp and females attracted from the natural population, and (2) blacklight lamp + virgin females + females attracted from the natural population. Westover Plantation, Rougon, Louisiana. September 19-October 4, 1970.

Type of Trap	Total Catch	Total Females	Total Males	Female to Male Ratio
1	300	24	276	1:11.5
2	341	35	306	1: 8.7
Total	641	59	582	1: 9.8

Table 7. Mating frequency and physiological age of female sugarcane borer moths attracted to traps baited with (1) blacklight lamps + females attracted from natural population, and (2) blacklight lamps + virgin females + females attracted from natural populations. Westover Plantation, Rougon, La. September 19-October 4, 1970.

Type of trap	<u>Mating Frequency</u>				<u>Physiological Age</u>		
	<u>Spermatophores</u>				<u>Group</u>		
	0	1	2	3	1	2	3
1	1	14	7	2	14	9	1
2	1	18	14	2	19	15	1
Total	2	32	21	4	33	24	2
%	3.4	54.2	35.5	6.8	55.9	40.7	3.4

Table 8. Total number of females of the sugarcane borer moth with indicated number of spermatophores attracted to blacklight traps classified by physiological age. Westover Plantation, Rougon, Louisiana. September 19-October 4, 1970.

Number of Spermatophores	Number of females with indicated number of spermatophores	Number of females in indicated physiological group		
		Group 1	Group 2	Group 3
0	2	2	0	0
1	32	21	10	1
2	21	9	11	1
3	4	1	3	0
Total	59	33	24	2
Percent		55.9	40.7	3.4

EXAMINATION AND THESIS REPORT

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Major Field: Entomology

Title of Thesis: Sex Pheromone and Blacklight Trap Studies With the Sugarcane Borer, Diatraea saccharalis (F.)

Approved:

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